

Symposium: Membrane Transport in Fatty Acid Synthesis and Obesity

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Sodium-Dependent Dicarboxylate and Citrate Transporters of the SLC13 Family

Ana M. Pajor.

University of California, San Diego, La Jolla, CA, USA.

The SLC13 family in humans and other mammals consists of sodium-coupled transporters for anionic substrates: three transporters for dicarboxylates/citrate and two transporters for sulfate. This presentation will focus on the di- and tricarboxylate transporters, NaDC1 (SLC13A2), NaDC3 (SLC13A3) and NaCT (SLC13A5). The substrates of these transporters are metabolic intermediates of the citric acid cycle, including citrate, succinate and α -ketoglutarate, which can exert signaling effects through specific receptors or affect metabolic enzymes directly. The SLC13 transporters are important for regulating plasma, urinary and tissue levels of citric acid cycle intermediates. NaDC1, primarily found on the apical membranes of renal proximal tubule and small intestinal cells, is important for regulating urinary levels of citrate and plays a role in kidney stone development. NaDC3 has a wider tissue distribution and high substrate affinity compared with NaDC1. NaDC3 participates in drug and xenobiotic excretion through interactions with organic anion transporters. NaCT is primarily a citrate transporter located in the liver and brain, and its activity may regulate metabolic processes. The recent crystal structure of the *Vibrio cholerae* homolog, VcINDY, provides a new framework for understanding the mechanism of transport in this family. Our structure-function studies with the mammalian transporters have identified residues that are important in binding substrates and cations. This presentation will summarize current knowledge of the structure, function and regulation of the di- and tricarboxylate transporters of the SLC13 family.

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Structure and Mechanism of a Bacterial Sodium-Dependent Dicarboxylate Transporter

Romina Mancusso¹, Da-Neng Wang².

¹New York Univ Med Ctr, New York, NY, USA, ²Skirball, New York Univ Med Ctr, New York, NY, USA.

In liver and adipose cells, cytosolic citrate is a major precursor for the synthesis of fatty acids, triacylglycerols, cholesterol and low-density lipoprotein. The cytosolic citrate concentration partially depends on direct import across the plasma membrane via the Na⁺-dependent citrate transporter (NaCT), a member of the divalent anion/Na⁺ symporter family (1,2). Mutations of the homologous transporter gene in flies (INDY, I'm not dead yet) result in reduced fat storage through calorie restriction (3). NaCT-knockout mice are both slimmer and protected from obesity and insulin resistance (4). We have determined the 3.2 Å crystal structure an INDY homolog from *Vibrio cholerae* (5). The protein consists of two halves that are related by an inverted twofold symmetry. One citrate molecule and one sodium ion are bound per protein, and their binding sites are formed by highly-conserved amino acid sequence motifs. Comparison of the structures of the two symmetrical halves of the transporter suggests conformational changes that propel substrate translocation.

1. E.M. Wright, *Ann Rev Physiol* 47, 127 (1985).
2. A.M. Pajor, *Pflug Arch Eur J Phy* 451, 597 (2006).
3. B. Rogina, R.A. Reenan, S.P. Nilsen, S.L. Helfand, *Science* 290, 2137 (2000).
4. A.L. Birkenfeld et al., *Cell Metab* 14, 567 (2011).
5. R. Mancusso, G.G. Gregorio, Q. Liu, D.N. Wang, *Nature* 491, 622 (2012).

78-Symp

I'm not Dead Yet: Flies and Mice

Andreas L. Birkenfeld¹, Varman T. Samuel², Gerald I. Shulman², Rafael De Cabo³, Robert A. Reenan⁴, Chen-Tseh Zhu⁴, Stephen Helfand⁴.

¹Center for Cardiovascular Research, Berlin, Germany, ²Yale School of Medicine, New Haven, CT, USA, ³National Institute on Aging, Baltimore, MD, USA, ⁴Brown University, Providence, RI, USA.

A decrease in the activity of the Indy gene (I'm not dead yet) in flies extends life span without a decline in fertility, physical activity, flight velocity or metabolic rate. Naturally occurring variants in Indy, worldwide, modify Indy expression and increase reproduction and longevity, indicating that alterations in Indy expression in the wild imparts significant improvement in fitness and longevity. The Indy gene codes for a high-affinity dicarboxylate/citrate plasma membrane transporter that is found most abundantly at the plasma membrane of adult fat body, oenocytes and midgut cells, the

primary sites of intermediary metabolism in the fly. Decreases in Indy affect metabolism similarly to calorie restriction (CR). CR down-regulates Indy in normal flies, and Indy long-lived flies share phenotypes with long-lived CR flies that include decreased insulin-like signaling, lipid storage, and weight, as well as resistance to starvation and increased spontaneous physical activity.

Knock-out of a mammalian homolog of Indy (mINDY; SLC13A5) in the mouse protects mice from age-related or high-fat-feeding-related insulin resistance and adiposity, and promotes phenotypes similar to those seen in CR. mINDY-deleted mice show increased hepatic mitochondrial biogenesis, lipid oxidation, and energy expenditure, as well as decreased hepatic de novo lipogenesis, activation of hepatic AMPK, induction of PGC-1 α , inhibition of ACC-2, and reduction of SREBP-1c. mINDY-deleted mice have reduced body weight, and preserve normal insulin signaling during aging or on high-fat diets, indicating that some of the positive effects of Indy in flies can be extended to mammals. Indy's effect on mammalian energy metabolism suggests that Indy is a potential target for treatment of obesity and type 2 diabetes, and that understanding of its regulation could lead to discovery of new agents for extending healthy life span and provide valuable insight into the genetic mechanisms of normal aging.

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In Vivo NMR Studies on the Mechanism of Lipid-Induced Insulin Resistance in Humans

Gerald I. Shulman.

Internal Medicine, Howard Hughes Medical Institute/Yale University, New Haven, CT, USA.

Insulin resistance is a major factor in the pathogenesis of type 2 diabetes and the metabolic syndrome. In this lecture I will discuss recent nuclear magnetic resonance studies that have implicated ectopic lipid deposition in liver and skeletal muscle as a causal and unifying factor in the pathogenesis of insulin resistance associated with obesity, lipodystrophy, type 2 diabetes and the metabolic syndrome. I will also present results from recent human studies demonstrating an important role of intracellular diacylglycerol, as the molecular trigger for lipid-induced insulin resistance in liver through its activation of protein kinase C ϵ and in muscle through its activation of protein kinase C θ , which both result in reductions in proximal insulin signaling.

Symposium: Force Generation in Cell and Tissue Networks

80-Symp

Mechanosensing by Tropomyosin-Controlled Myosin Contractions

Michael Sheetz^{1,2}.

¹Columbia University, New York, NY, USA, ²Mechanobiology Institute, National University of Singapore, Singapore, Singapore.

Control of cell growth, death or differentiation involves the integration of microenvironmental signals through cell motile processes to produce the desired cellular responses. In the case of cell-matrix interactions, the cell tests the matrix by asking several if-then questions such as is the matrix clustered, can force be generated and later what is the rigidity of the matrix. Using lipid-linked matrix ligands, we found that cells would not spread on the surface unless the liganded integrins were clustered and cells generated forces on the ligands by pulling to barriers (Yu et al., 2011. PNAS 108:20565). Upon activation of cell spreading, the flattening of the cells removed the folds in the membranes until a rise in membrane tension activated contraction to sense rigidity (Gauthier et al., 2011. PNAS 108:14467). Rigidity sensing involved local contraction units that pulled about 100 nm independent of rigidity (Ghassemi et al., 2012 PNAS 109:5325). Recently, we found that local contraction units resembled dynamic sarcomeres with alpha actinin at ends and myosin in the middle (Meacci et al., submitted). Further, generation of force involved repeated step-wise movements of myosin controlled by tropomyosin-1 (Wolfenson et al., submitted). Angstrom level measurements of fibroblasts pulling on elastomeric PDMS pillars showed that pillar displacement occurred in discrete steps of ~1 nm. In contractile pairs, simultaneous steps of opposing pillars had a total step size of ~2.2 nm, independent of rigidity. Changes in the stepping patterns on different rigidities indicated that the level of contractile force was critical for sensing pillar stiffness. Importantly, knockdown of tropomyosin-1 caused larger steps and increased forces that resulted in aberrant rigidity sensing. This indicates that the process of cell-matrix adhesion formation involves multiple, sequential steps with if-then decisions based upon the physical as well as biochemical properties of the matrix.